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Multicenter clinical evaluation of alinity m HCV assay performance

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ABSTRACT

Background: Nucleic acid testing is essential for the detection and quantification of HCV RNA in the diagnosis of HCV infection and treatment monitoring. The Alinity m HCV assay was recently developed by Abbott Molecular for rapid detection and quantification of HCV RNA on the fully automated, continuous, random-access Alinity m analyzer.

Objectives: Our study assessed the performance of the new Alinity m HCV assay for detection and quantification of HCV RNA in a large series of patient samples of various genotypes. This international, multicentric study evaluated the linearity, precision, and reproducibility of the Alinity m HCV assay and its performance in comparison to three other HCV assays currently used in clinical practice.

Results: The Alinity m HCV assay demonstrated high linearity (correlation coefficient r = 1.00), precision (coefficients of variation [CV] 6.6–13.5 %) and reproducibility (CV 1.7–4.3 % across three control lots). At a concentration near the lower limit of detection, the Alinity m HCV assay exhibited > 98 % detectability. The Alinity m HCV assay showed excellent correlation with comparator HCV assays in serum (n = 406) and plasma (n = 1401) samples (correlation coefficients ≥0.96, bias 0.01 to 0.14 Log_{10} IU/mL). More than 95 % of the quantified results with the Alinity m HCV assay were less than mean bias ± 1.96 SD different from those of the comparator assays.

Conclusions: The newly developed Alinity m HCV assay is sensitive, reproducible, and accurately quantifies HCV RNA levels in serum and plasma samples from patients with chronic HCV infection, with no impact of HCV genotype on assay performance.

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1. Introduction

The World Health Organization (WHO) estimates that 71 million people worldwide are chronically-infected with hepatitis C virus (HCV), and nearly 600,000 individuals die each year from complications of HCV including primarily cirrhosis and hepatocellular carcinoma (HCC) [1]. Hepatitis C virus is the leading virus-related cause of HCC in North America, Europe, Japan, parts of central Asia, and northern Africa and the Middle East [2]. Despite the development of highly active, all-oral, well-tolerated, short-duration drugs with high cure rates (> 95 %), chronic HCV infection remains a global public health concern. The endpoint of therapy is sustained virologic response (SVR), defined as undetectable HCV RNA in serum or plasma 12 or 24 weeks after the treatment cessation. SVR is associated with reduced liver inflammation, fibrosis regression, and increased quality of life and overall survival.

For detection and quantification of HCV RNA in clinical practice, sensitive and accurate nucleic acid technologies (NATs) are recommended by international clinical practice guidelines [3,4]. HCV RNA assays are now widely used in clinical virology laboratories for both diagnosis and treatment monitoring, and are partly or fully automated. With a broad range of linear quantification [up to 10^8 international units per milliliter (IU/mL)], a limit of detection (LOD) in the order of 15 IU/mL or less, and an identical lower limit of quantification (LLOQ), NATs for HCV RNA are well suited to meet clinical needs [5].

The Alinity™ m HCV assay (Abbott Molecular, Des Plaines, IL, USA) is a polymerase chain reaction (PCR) dual-probe assay that makes use of the fully automated Alinity m system, which eliminates the need for batch processing of samples and automates all aspects of nucleic acid testing from sample loading to result reporting. With the Alinity m system, the first 12 HCV assay results are obtained in less than 2 h, with 12 additional results reported every 16 min thereafter. Here, we examined the performance of the new Alinity m HCV assay compared to three different commercially available HCV assays for accurate HCV RNA detection and quantification in a very large series of patients chronically infected with different HCV genotypes.

2. Materials and methods

2.1. Clinical specimens and study sites

Serum (n = 406) and plasma (n = 1401) samples from patients with chronic HCV infection were collected at nine health care sites in Europe, South Africa, UK and Australia (Azienda Ospedaliera di Padova, Padua, Italy; Hôpital Universitaire Henri Mondor, Créteil, France; Hospital Universitario Ramón y Cajal, Madrid, Spain; Laboratori de Referència de Catalunya, El Prat de Llobregat, Spain; Lancet Laboratories, Johannesburg, South Africa; Medizinisches Infektiologiezentrum Berlin, Germany; Laboratory Dr. Knechten, Aachen, Germany; Victorian Infectious Diseases Reference Laboratory, Melbourne, Australia; West of Scotland Specialist Virology Centre, Glasgow, UK).

Genotype information based on phylogenetic analysis of a portion of the NS5B gene using a deep sequencing-based assay (Sentosa® SQ HCV Genotyping Assay, Vela Diagnostics, Singapore) or an in-house Sanger method as previously described [6] was available for 375 clinical specimens: 214 genotype 1, 35 genotype 2, 59 genotype 3, 40 genotype 4, 2 genotype 5, and 10 genotype 6. The remaining 15 clinical samples were from patients infected with a 2k/1b (n = 7) or 1b/3a (n = 3) recombinant strain or a mixed 1a plus 3a infection (n = 5).

The study was performed in accordance with the principles of Good Clinical Practice and conducted in adherence with the Declaration of Helsinki. Only residual samples for routine HCV testing were used. All samples were anonymized by assigning an identification number without any patient information to each sample prior to the start of the study.

2.2. HCV RNA detection and quantification

Clinical specimens were tested with the Alinity m HCV assay and the commercially available HCV assay used for routine testing at each study site. Comparator assays were based on either real-time PCR [RealTi*m*e HCV (Abbott Molecular) or cobas® HCV Test (Roche Molecular Systems, Pleasanton, CA)] or transcription-mediated amplification [TMA; Aptima® HCV Quant Dx assay (Hologic Inc., San Diego, CA)].

Alinity m HCV assay: In a fully automated process, HCV RNA was isolated on the Alinity m System from 600 µL of plasma or serum according to the manufacturer's instructions. The fluorescence curves were analyzed by the Alinity m system software, versions 1.2, 1.3, or 1.3.1. The dynamic range of quantification of the Alinity m HCV assay is 12 to 1×10^8 IU/mL (1.1–8.0 Log_{10} IU/mL) with an LOD of 5.11 IU/mL in plasma and serum.

RealTi*m*e HCV assay: HCV RNA was processed from 500 µL of plasma or serum using the *m*2000sp™ and the *m*2000rt analyzers for automated sample extraction and real-time PCR amplification and detection, according to the manufacturer's instructions. The data were analyzed with Abbott RealTi*m*e *m*2000rt software, version 8.1. The dynamic range of quantification of this assay is 12 to 1×10^8 IU/mL (1.1–8.0 Log_{10} IU/mL), with an LOD of 10.5 IU/mL in plasma and 7.2 IU/mL in serum.

cobas HCV assay: HCV RNA was extracted, purified and amplified from 500 µL of plasma or serum on the cobas 6800 System according to the manufacturer's instructions (Roche Molecular Systems). The dynamic range of quantification is 15 to 1×10^8 IU/mL (1.2–8.0 Log_{10} IU/mL), with an LOD of 8.5 IU/mL in plasma and 9.6 IU/mL in serum.

Aptima HCV Quant Dx assay: HCV RNA was isolated from 500 µL of plasma using the Panther® analyzer system according to the manufacturer's instructions. The dynamic range of quantification of Aptima HCV Quant Dx is 10 to 1×10^8 IU/mL (1.0–8.0 Log_{10} IU/mL), with an LOD of 4.3 IU/mL in plasma.

The conserved 5′ untranslated region (5′ UTR) is the target region of the primers and probes in all four assays, respectively.

2.3. Analytical assessment of alinity m HCV – precision, detectability and reproducibility

Assay precision and detectability were evaluated across 4 study sites by using a non-commercial panel, consisting of different concentrations of HCV RNA and generated by dilution of HCV-positive clinical samples in normal human plasma (Exact Diagnostics, Fort Worth, TX, USA). Each panel contained a predetermined amount of HCV RNA adjusted based on the RealTi*m*e™ HCV assay (Abbott Molecular). The nine panel members contained target concentrations of 1×10^2 IU/mL (2.00 Log_{10} IU/mL), 25 IU/mL (1.40 Log_{10} IU/mL) and 10 IU/mL (1.00 Log_{10} IU/mL) for each HCV RNA genotype 1a, 1b, and 3a, respectively. Mean, standard deviation (SD) and percent coefficient of deviation (% CV) were evaluated at concentrations > 10 IU/mL and detectability was evaluated at 10 IU/mL.

Reproducibility was assessed by evaluating assay controls from multiple reagent lots used across all testing sites. Overall mean, SD and %CV of each assay's quality controls (QC) were evaluated.

2.4. Statistical analysis

Descriptive statistics are reported as means ± SD. Relationships between quantitative variables were analyzed by Deming regression. Differences in quantification between the assays were evaluated by Bland Altman analysis. All statistical analyses were performed using PC SAS version 9.3 (SAS, Cary, NC, USA).

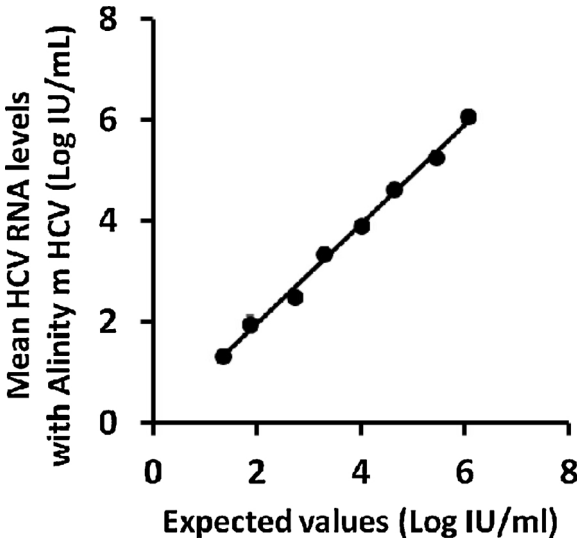

Fig. 1. Alinity m HCV assay linearity. HCV RNA quantification by the Alinity m HCV assay of a dilution series of a clinical serum specimen containing 8.30×10^6 IU/mL (6.90 Log_{10} IU/mL) HCV RNA.

3. Results

3.1. Linear quantification by Alinity m HCV

The linearity of Alinity m HCV assay was assessed by testing serial dilutions of a serum specimen collected from a patient infected with genotype 2, which contained 8.30×10^6 IU/mL (6.90 Log_{10} IU/mL) HCV RNA. To prepare 8 diluted concentrations, with the lowest one near the LLOQ of the assay, a serial dilution with a dilution factor of 4.2 was performed. Each dilution was tested in triplicate in the same run. Means and ranges were compared with the theoretical HCV RNA levels. The expected difference in quantitation between the concentration levels was 0.62 Log_{10} IU/mL. The observed differences between subsequent dilutions were comparable and compatible with the expected values (0.81 ± 0.12; 0.62 ± 0.11; 0.72 ± 0.06; 0.57 ± 0.00; 0.85 ± 0.05; 0.53 ± 0.17; 0.64 ± 0.08 Log_{10} IU/mL). Fig. 1 shows the measured values obtained with the serial dilutions compared to the expected values (correlation coefficient, r = 1.00, linear regression equation, y = 0.9858 x + 0.0016).

3.2. Precision, detectability and reproducibility of Alinity m HCV

Precision was evaluated by testing a panel consisting of dilutions of clinical samples with 100 IU/mL (2.00 Log IU/mL) and 25 IU/mL (1.40 Log IU/mL) HCV RNA of genotype 1a, 1b, or 3a. As shown in Table 1, the coefficients of variation varied from 6.6% to 13.5% for Alinity m. At a concentration near the LLOD, the Alinity m HCV assay exhibited a high level of detectability (> 98 %). Reproducibility was characterized by a coefficient of variation of 4.3 % for the low positive control (LPC) and of 1.7 % for the high positive control (HPC) across the three different HCV control lots used during the study (Table 1).

Table 2
Comparison of HCV RNA Levels Using the Alinity m HCV and Abbott RealTime HCV Assays.

		Abbott RealTime HCV (n)		
		Not detected	< 12 IU/mL	≥12 IU/mL
Alinity m HCV (n)	**Not detected**	342	160	0
	< 12 IU/mL	20	64	2[a]
	≥12 IU/mL	8[b]	24[c]	883*

* One additional sample was above the upper limit of quantification (8.00 Log_{10} IU/mL) for both methods. Another additional sample was > 8.00 Log_{10} IU/mL with Alinity m but quantified with Abbott RealTime HCV.
[a] RealTime results ranged from 1.28 to 1.40 Log_{10} IU/mL.
[b] Alinity m results ranged from 1.09 to 1.93 Log_{10} IU/mL.
[c] Alinity m results ranged from 1.09 to 1.80 Log_{10} IU/mL.

3.3. Clinical performance of Alinity m HCV assay

In total, 1807 samples from HCV-seropositive individuals were retrospectively tested with Alinity m HCV assay at the nine participating study sites. Results were compared to previous routine test results obtained by using one of the three comparator assays: Abbott RealTime HCV assay (n = 1505), cobas HCV assay (n = 186), and Aptima HCV Quant Dx assay (n = 116).

Of the 1505 samples tested by the Alinity m HCV and Abbott RealTime HCV assays, 883 fell within the dynamic range of both assays (Table 2). Fig. 2 and Table 3 show the relationships between HCV RNA levels quantified with both assays. A strong correlation between the two assays was found (r = 0.98; Deming regression equation, y = 1.00 x + 0.16). A weak bias across the quantitative range of the two assays was observed using Bland Altman analysis (0.13 ± 0.28 Log_{10} IU/mL; Table 3), indicating no major difference in quantification according to the HCV RNA level. In 95.4 % (842/883) samples, the difference between the assays was within ± 1.96 times the SD of the mean bias. Of the remaining samples, 1.7 % (15/883) were measured higher and 2.9 % (26/883) measured lower with the Alinity m HCV assay.

Of the 186 samples tested by the Alinity m HCV and cobas HCV assays, 163 fell within the dynamic range of both assays (Table 4). A strong correlation between the two assays was found (r = 0.96; Deming

Table 1
Precision, Detection Rates, and Reproducibility of the Alinity m HCV Assay.

	Panel Member (Genotype) / Control	Target HCV RNA* (Log_{10} IU/mL)	No. of Replicates	Mean of Measured HCV RNA (Log_{10} IU/mL)	SD (Log_{10} IU/mL)	CV (%)	No. of Replicates Detected	Detection Rate (%)
Precision	GT 1a-1	2.00	58	2.05	0.17	8.1	–	–
	GT 1a-2	1.40	54	1.38	0.16	11.9	–	–
	GT 1b-1	2.00	56	2.23	0.15	6.6	–	–
	GT 1b-2	1.40	59	1.54	0.21	13.5	–	–
	GT 3a-1	2.00	56	2.24	0.17	7.5	–	–
	GT 3a-2	1.40	56	1.65	0.16	9.8	–	–
Detection Rate	GT 1a-3	1.00	58	–	–	–	57	98.3
	GT 1b-3	1.00	57	–	–	–	57	100.0
	GT 3a-3	1.00	60	–	–	–	60	100.0
Reproducibility	LPC	2.71 – 2.81	215	2.70	0.12	4.3	–	–
	HPC	6.02 – 6.23	215	6.06	0.11	1.7	–	–

SD: standard deviation; CV: coefficient of variation; GT: genotype; LPC: low positive control; HPC: high positive control.
* For LPC and HPC, the range reflects the different target concentrations across the control lots used during the study.

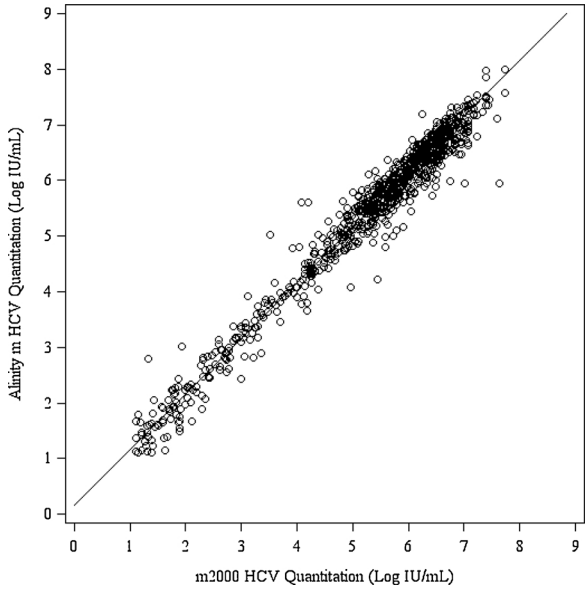

Fig. 2. Alinity m HCV assay and Abbott RealTi*m*e HCV assay performance. Deming regression of HCV RNA levels showing correlation between the Alinity m HCV assay and Abbott RealTi*m*e HCV assay.

regression equation, y = 0.90 x + 0.48; Fig. 3, Table 3). Bland Altman analysis resulted in a negligible bias across the quantitative range of the two assays (-0.01 ± 0.45 Log_{10} IU/mL; Table 3). No major difference in quantification was observed according to the HCV RNA level. In 94.1 % (175/186) samples, the difference between the assays was within ± 1.96 times the SD of the mean bias. Of the remaining samples, 4.8 % (9/186) were higher and 1.1 % (2/186) lower with the Alinity m HCV assay.

Of the 116 samples tested by the Alinity m HCV and Aptima HCV Quant Dx assays, 18 fell within the dynamic range of both assays (Table 5). A strong correlation between the two assays was found (r = 0.99; Deming regression equation, y = 1.02 x + 0.02; Fig. 4, Table 3). A weak bias of HCV RNA level was observed by Bland Altman analysis across the quantitative range of the two assays (0.14 ± 0.23 Log_{10} IU/mL; Table 3). No major difference of quantification was observed according to the HCV RNA level as all samples showed a difference between the assays within ± 1.96 times the SD of the mean bias.

3.4. *Influence of HCV genotype on HCV RNA quantification*

The influence of the HCV genotype on HCV RNA quantification was assessed by comparing results of the Alinity m HCV and Abbott RealTi*m*e HCV assays of a subset of serum and plasma samples from 375 patients infected with HCV genotypes 1–6. A strong relationship was found between HCV RNA levels measured in the same specimens with the Alinity m HCV assay and Abbott RealTime HCV assay, regardless of the HCV genotype (data not shown).

Table 4
Comparison of HCV RNA Levels Using the Alinity m HCV and cobas HCV Assays.

		cobas HCV (n)		
		Not detected	< 15 IU/mL	≥15 IU/mL
Alinity m HCV (n)	**Not detected**	17	3	0
	< 12 IU/mL	1	1	0
	≥12 IU/mL	0	1[a]	163

[a] Alinity m sample quantitated at 1.72 Log_{10} IU/mL.

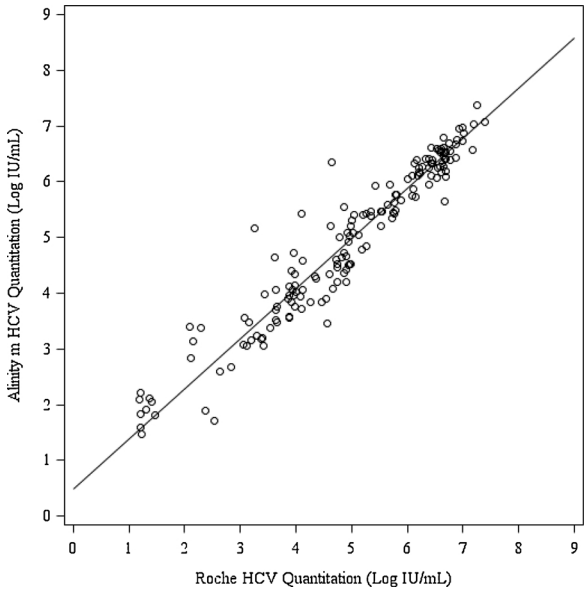

Fig. 3. Alinity m HCV assay and cobas HCV assay performance. Deming regression of HCV RNA levels showing correlation between the Alinity m HCV assay and cobas HCV assay.

Table 5
Comparison of HCV RNA Levels Using the Alinity m HCV and Aptima HCV Quant Dx Assays.

		Aptima HCV Quant Dx (n)		
		Not detected	< 10 IU/mL	≥10 IU/mL
Alinity m HCV (n)	**Not detected**	44	50	0
	< 12 IU/mL	0	4	0
	≥12 IU/mL	0	0	18

4. Discussion

In this international multicenter study evaluating a large number of clinical specimens, we showed that the new Alinity m HCV assay accurately quantifies HCV RNA levels. Results indicated that there is no impact of the HCV genotype on assay performance based on previous studies [7]. The performance of the Alinity m HCV assay was comparable to several HCV assays that are currently widely used in clinical practice, including the Abbott RealTi*m*e HCV assay and the cobas HCV

Table 3
Performance Comparison of Alinity m HCV and Comparator HCV Assays.

Comparator Assay	Quantified Samples (n)	Correlation Coefficient (r)	Mean of Bias (Alinity m - Comparator; Log_{10} IU/mL)	SD of Bias (Log_{10} IU/mL)
Abbott RealTi*m*e HCV	883	0.983	0.13	0,283
cobas HCV	163	0.959	−0.01	0.454
Aptima HCV Quant Dx	18	0.991	0.14	0.232

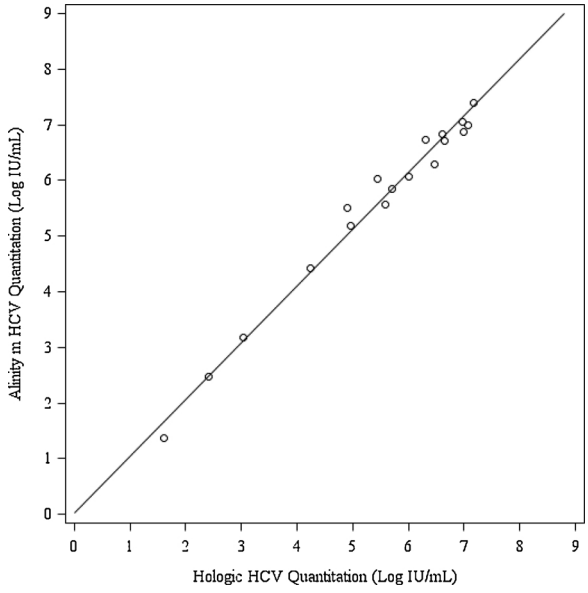

Fig. 4. Alinity m HCV assay and Aptima HCV Quant Dx assay performance. Deming regression of HCV RNA levels showing correlation between the Alinity m HCV assay and Aptima HCV Quant Dx assay.

assay.

An excellent correlation was found between the Alinity m HCV assay and comparator HCV assays, with correlation coefficients ≥0.96. We observed only a low difference in HCV RNA levels measured with the Alinity m HCV assay, with an overall bias ranging from -0.01 to 0.14 Log_{10} IU/mL. Overall, more than 95 % of the quantifiable patient results with the Alinity m HCV assay were less than ± 1.96 SD of the mean bias different from the respective comparator assays (94 %–100 %). The observed low differences in performance will most likely have no implications for clinical practice.

A larger number of specimens was observed with detectable HCV RNA < LOQ by either Abbott RealTi*m*e HCV or Aptima HCV Quant Dx compared to undetectable HCV RNA results with Alinity m HCV (Tables 2 and 5). One hypothesis will be additional free/thaw cycles of specimens prior to testing with Alinity m HCV compared to testing with Abbott RealTi*m*e HCV. A higher detection rate obtained with Aptima HCV compared to Alinity m HCV could be due to the lower LOQ of 10 IU/mL of the Aptima HCV assay compared to 12 IU/mL of the Alinity m HCV assay.

This study has several limitations. The small proportion of clinical specimens containing genotypes 5 and 6 tested reflects the HCV genotype distribution in Europe. Thus, although our results strongly suggest that the performance of the Alinity m HCV assay will be high for all genotypes, further studies are warranted. The utility of the Alinity m HCV assay for longitudinal HCV RNA monitoring in patients receiving direct-acting antiviral (DAA) therapy is also not known. Unfortunately, no clinical data were available and the proportion of clinical samples collected from patients receiving antiviral treatment was unknown. However, the large number of clinical specimens (16.9 %) with detectable but nonquantifiable HCV RNA by any test were probably collected in patients receiving antiviral therapy (week 4 or end of therapy). Finally, although we tested more than 100 samples with the Aptima HCV Quant Dx assay, the number of samples quantified with both Alinity m and Aptima HCV assays was relatively small (18 samples).

In conclusion, with this first field evaluation of the Alinity m HCV assay, we showed that the newly developed real-time PCR-based Alinity m HCV assay is sensitive, reproducible, and accurately quantifies HCV RNA levels in serum and plasma samples from patients with chronic HCV infection. Quantification is linear across the full dynamic range, covering values observed in untreated and DAA-treated patients. In addition, the Alinity m platform is fully automated with a consolidated, continuous, and random-access workflow. With its processing capacity of 300 samples per 8 -h shift and the capability to immediately process urgent (STAT) samples, it enables same-day reporting of HCV test results. Rapid diagnostic testing can shorten the time between diagnosis and treatment, which may improve patient care and outcomes. The Alinity m HCV assay can thus confidently be used to detect and quantify HCV RNA in clinical practice.

CRediT authorship contribution statement

Stéphane Chevaliez: Methodology, Formal analysis, Resources, Writing - original draft, Writing - review & editing, Visualization. **Francesco Onelia:** Methodology, Validation, Investigation, Resources, Data curation, Writing - review & editing. **Monia Pacenti:** Methodology, Resources, Writing - review & editing. **Emily Goldstein:** Validation, Investigation, Data curation, Writing - review & editing. **Juan-Carlos Galán:** Methodology, Resources, Writing - review & editing. **Laura Martínez-García:** Validation, Investigation, Data curation, Writing - review & editing. **Alba Vilas:** Methodology, Resources, Writing - review & editing. **Allison Glass:** Methodology, Resources, Writing - review & editing. **Leana Maree:** Methodology, Resources, Writing - review & editing. **Maria Krügel:** Validation, Investigation, Data curation, Writing - review & editing. **Robert Ehret:** Methodology, Validation, Investigation, Data curation, Writing - review & editing. **Heribert Knechten:** Methodology, Resources. **Patrick Braun:** Methodology, Resources, Writing - review & editing. **Gudrun Naeth:** Methodology, Validation, Investigation, Data curation, Writing - review & editing. **Sara Bonanzinga:** Validation, Investigation, Data curation, Writing - review & editing. **Kathy Jackson:** Methodology, Resources, Writing - review & editing. **Klara Abravaya:** Conceptualization, Methodology, Resources. **Jens Dhein:** Conceptualization, Methodology, Data curation, Writing - review & editing. **Shihai Huang:** Conceptualization, Methodology, Resources, Writing - review & editing. **Ajith M. Joseph:** Conceptualization, Methodology, Data curation, Writing - review & editing. **Danijela Lucic:** Conceptualization, Methodology, Formal analysis, Data curation, Writing - review & editing. **Natalia Marlowe:** Conceptualization, Methodology, Writing - review & editing. **Michael J. Palm:** Conceptualization, Methodology. **Karin Pfeifer:** Conceptualization, Methodology, Data curation, Writing - review & editing. **Dan Toolsie:** Methodology, Resources. **Birgit Reinhardt:** Conceptualization, Methodology, Formal analysis, Data curation, Writing - original draft, Writing - review & editing, Visualization. **Martin Obermeier:** Methodology, Resources, Writing - review & editing. **Rory Gunson:** Methodology, Resources, Writing - review & editing.

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